UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 30, 2008

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No.777-99, Brace;
DP Barcode: 355134

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)
11/4/08

Thru: Michele Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Adam Heyward PM 34/ Renae Whitaker
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser, Inc.
Morris Corporate Center IV
399 Interspace Parkway
PO Box 225
Parsippany, NJ 07054-0225

Formulations from Label

Active Ingredient(s)	% by wt.
Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) Dimethyl benzyl ammonium chloride	0.10%
Ethanol	58.00%
Other Ingredients	41.90%
Total	100.00%

I BACKGROUND

The product, Brace (EPA Reg. No. 777-99), is registered disinfectant (bactericide, fungicide, tuberculocide, virucide), non-food contact surface sanitizer, mildewstat, and deodorizer for use on hard, non-porous surfaces in household, institutional, industrial, commercial, animal care, and hospital or medical environments. The product is an aerosol. This data package provided confirmatory data for a number of alternate formulations. This data package also included efficacy data to support use of the product as a soft surface, non-food contact sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and at Reckitt Benckiser Inc., Microbiology Laboratory, located at One Philips Parkway, in Montvale, NJ 07645.

This data package contained a letter from the applicant to EPA (dated July 10, 2008), a letter from EPA to the applicant (dated June 10, 2008), EPA Form 8570-27 (Formulator's Exemption Statement), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), seven studies (MRID 474759-01 through -07), Statements of No Data Confidentiality Claims for all seven studies, the proposed label, and the last accepted label (dated June 12, 2008).

II USE DIRECTIONS

The product is designed to be used for disinfecting and sanitizing hard, non-porous surfaces such as appliance exteriors, bathtubs, bed frames and bed springs, cabinets, cat litter boxes, carts, counter tops, cuspidors, furniture, diaper changing tables, diaper pails, dish pails and racks, door knobs, drinking fountains, examination tables, faucets, fixtures, floors, garbage cans, lamps, laundry baskets, light switches, mattress covers, metal blinds, mirrors, outdoor furniture, recycling bins, remote controls, showers, sinks, sports equipment, stretchers, toilets, toys, telephones, tools, urinals, walls, wheelchairs, whirlpool interiors, and windows. The proposed label indicated that the product can be used on surfaces such as crystal, enamel, glass, glazed ceramic tile, glazed porcelain, laminate, linoleum, marble, Marlite, metal (e.g., brass, chrome, copper, stainless steel, tin), Parquet, plastic, Plexiglas, sealed granite, and vinyl. Directions on the proposed label provided the following information regarding use of the product:

> As a disinfectant: Pre-clean surfaces prior to use. Hold container upright 6-8 inches from surface. Spray for 2-3 seconds until covered with mist. Let stand for 10 minutes to air dry. Food contact surfaces must be thoroughly rinsed with potable water.
>
> As a soft (fabric) surface sanitizer: Spray a light, even coating on fabric until wet. Fabric must remain wet for 30 seconds. Do not saturate. Let air dry. For difficult odors or heavy fabrics, repeat application.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Confirmatory Efficacy Data Requirements)

Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formation. This includes a minor formulation change (e.g., a change in an inert ingredient) in a registered product. Confirmatory data must be developed on the applicant's own finished product. For hospital disinfectants, 10 carriers on each of 2 samples representing 2 different product lots must be tested against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Killing on all carriers is required.

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. Testing requirements in EPA DIS/TSS-10 and/or ASTM E1153-94 may be used. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. Furthermore, according to information provided in Section 12.3.2 of ASTM E1153-94, which is a test method for the efficacy of sanitizers for non-food contact surfaces, "an average of at least 7.5 x 10^5 organisms must have survived on the inoculated control squares for the test to be valid."

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 474759-01 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Surface Non-Food Contact Surfaces (Modified for Spray Product Application)," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) for Formula Number 677-180, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – November 7, 2005. Study Identification Number A03070.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Three lots (Lot Nos. 960-028, 960-030, and 960-032) of the product, Formula Number 677-180, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use. Cultures of the challenge microorganisms were

prepared in accordance with published AOAC methods with the following exception: cultures were allowed to stand at least 15 minutes after vortex mixing (which is a greater amount of time than the 10 minutes specified in the AOAC methods). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. The carriers for this test were prepared from plain cotton weave fabric (approximately 80 x 80 threads/inch). A scouring solution was prepared by adding 1.5 grams Na_2CO_3, 1.5 grams of Triton X-100, and 3 L of deionized water. The fabric (284.1 grams) was added to 3 L of the scouring solution and boiled for 60 minutes. The fabric then was rinsed for 5 minutes in boiling water and 5 minutes in cold water. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. The fabric was allowed to air dry. Five 1 inch x 1 inch steam-sterilized fabric carriers per product lot per microorganism were inoculated with 0.02 mL of a 48 hour and 30 minute old suspension of the test organism. The carriers were dried for 40 minutes at 36.0°C at 40% relative humidity in a constant humidity chamber. The carriers were removed to room temperature. Each carrier was sprayed for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 30 seconds at 21°C. Following exposure, individual carriers were transferred to jars containing 20 mL of Letheen Broth with 0.07% Lecithin, 0.5% Tween 80, and glass beads. Each jar was rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes after neutralizing, 1.0 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All plates were incubated for 45 hours and 30 minutes at 36°C. Subcultures were stored for 22 hours at 2-8°C prior to examination. Following incubation and storage, the colonies were counted. Controls included those for purity, sterility, viability, carrier quantitation (i.e., parallel control), dry carrier count, inoculum count, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 474759-02 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Surface Non-Food Contact Surfaces (Modification of Spray Product Application)," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) for Formula Number 1056-010B, Formula Number 1056-054A, and Formula Number 1056-010C, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – January 28, 2008. Study Identification Number A05514.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Three lots (Lot Nos. 1056-078, 1056-079, and 1056-080) corresponding to three products, Formula Number 1056-010B, Formula Number 1056-054A, and Formula Number 1056-010C, respectively, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). Each of the product lots tested was at least 60 days old at the time of testing. The product was received ready-to-use. Testing was conducted on November 13, 2007 and December 11, 2007. Cultures of the challenge microorganisms were prepared in accordance with published AOAC methods with the following exception: cultures were allowed to stand at least 15 minutes after vortex mixing (which is a greater amount of time than the 10 minutes specified in the AOAC methods). Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. The carriers for this test were prepared from a polyester/acrylic blend of fabric. A scouring

solution was prepared by adding 1.5 grams Na_2CO_3, 1.5 grams of Triton X-100, and 3 L of deionized water. The fabric (~100 grams) was added to 1 L of the scouring solution (or the equivalent) and boiled for 60 minutes. The fabric then was rinsed for 5 minutes in boiling water and 5 minutes in cold water. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. The fabric was allowed to air dry. Five 1 inch x 1 inch steam-sterilized fabric carriers per product lot per microorganism were inoculated with 0.02 mL of a 48±4 hour suspension of the test organism. The carriers were dried for 20 minutes at 35-37°C at 39-40% relative humidity in a constant humidity chamber (which is a shorter time than the 40 minutes specified in the DIS/TSS-10 method). The carriers were removed to room temperature. Each carrier was sprayed for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 30 seconds at 21-22°C. Following exposure, individual carriers were transferred to jars containing 20 mL of Letheen Broth with 0.14% Lecithin, 1.0% Tween 80, and glass beads. Each jar was rotated vigorously on an even plane for ~50 rotations to suspend the surviving organisms. Within 30 minutes after neutralizing, 1.0 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All plates were incubated for 48±4 hours at 35-37°C. Following incubation, the colonies were counted. Controls included those for purity, sterility, viability, carrier quantitation, dry carrier count, inoculum count, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing performed on November 13, 2007 did not demonstrate expected efficacy results for Lot No. 1056-080 of Formula Number 1056-010C against *Staphylococcus aureus*. Testing was repeated on December 11, 2007. See pages 8 and 11 and Tables 6 and 7 of the laboratory study.

3. MRID 474759-03 "Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil" for Formula Number 1178-172, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – June 20, 2008. Study Identification Number 2008-0033.

This confirmatory study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 1325-046 and 1325-069) of the product, Formula Number 1178-172, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Testing was conducted on April 4, 2008 and April 30, 2008. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods with the following exception: final cultures were coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot per microorganism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The inoculum was spread evenly over the carrier surface. The carriers were dried for 40-42 minutes at 33.6-34.7°C (which is a slightly longer time than the 30-40 minutes specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Each carrier was sprayed with the product for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 5 minutes at 22.0-22.5°C. Following exposure,

individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures were gently shaken (see protocol) as specified in the AOAC method. For testing conducted on April 4, 2008, all subcultures were incubated for at least 70 hours at 33.3-34.9°C (which is a longer time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). For testing conducted on April 30, 2008, all subcultures were incubated for 46 hours and 45 minutes at 33.3-34.8°C (which is a slightly shorter time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery carrier count, test system verification (i.e., purity and identity), neutralizer efficacy, and sterility.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing performed on April 4, 2008 did not demonstrate expected efficacy results for Lot No. 1325-046 against *Staphylococcus aureus* and Lot No. 1325-069 against *Pseudomonas aeruginosa*. On April 30, 2008, testing was repeated to test for potential false positives. See page 15 and Appendix C of the laboratory study.

4. MRID 474759-04 "Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil" for Formula Number 1338-027, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – June 20, 2008. Study Identification Number 2008-0034.

This confirmatory study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 1325-045 and 1325-047) of the product, Formula Number 1338-027, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods with the following exception: final cultures were coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot per microorganism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The inoculum was spread evenly over the carrier surface. The carriers were dried for 40-42 minutes at 35.0-35.9°C (which is a slightly longer time than the 30-40 minutes specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Each carrier was sprayed with the product for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 5 minutes at 22.8-22.9°C. Following exposure, individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures were gently shaken (see protocol) as specified in the AOAC method. All subcultures were incubated for at ~48 hours at 34.5-35.6°C (which is a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery carrier count, test system verification (i.e., purity and identity), neutralizer efficacy, and sterility.

5. MRID 474759-05 "Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil" for Formula Number 1338-016, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – June 25, 2008. Study Identification Number 2008-0063.

This confirmatory study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 1367-073 and 1367-076) of the product, Formula Number 1338-016, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods with the following exception: final cultures were coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot per microorganism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The inoculum was spread evenly over the carrier surface. The carriers were dried for 40 minutes at 33.1-34.0°C (which is a slightly cooler temperature than the 37°C specified in the AOAC method). Each carrier was sprayed with the product for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 5 minutes at 22.3-23.0°C. Following exposure, individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures were gently shaken (see protocol) as specified in the AOAC method. All subcultures were incubated for 46 hours and 41 minutes at 33.7-35.7°C (which is a slightly shorter time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery carrier count, test system verification (i.e., purity and identity), neutralizer efficacy, and sterility.

6. MRID 474759-06 "Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil" for Formula Number 1338-015, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – June 20, 2008. Study Identification Number 2008-0064.

This confirmatory study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 1367-064 and 1367-068) of the product, Formula Number 1338-015, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods with the following exception: final cultures were coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot per microorganism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The inoculum was spread evenly over the carrier surface. The carriers were dried for 41 minutes at 33.1-34.0°C (which is a slightly longer time than the 30-40 minutes specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Each carrier was sprayed with the product for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 5 minutes at 22.6-23.0°C. Following exposure, individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures were gently shaken (see protocol) as specified in the AOAC method. All subcultures were incubated for 46 hours and 10 minutes at

33.7-35.7°C (which is a slightly shorter time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery carrier count, test system verification (i.e., purity and identity), neutralizer efficacy, and sterility.

7. MRID 474759-07 "Hospital Type Disinfectant Efficacy Testing in the Presence of Organic Soil" for Formula Number 1338-020, by Kyle T. Smith. Study conducted at Reckitt Benckiser Inc. Study completion date – June 25, 2008. Study Identification Number 2008-0065.

This confirmatory study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Two lots (Lot Nos. 1367-080 and 1367-083) of the product, Formula Number 1338-020, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Testing was conducted on April 23, 2008 and May 7, 2008. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods with the following exception: final cultures were coarse filtered. Horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per product lot per microorganism were inoculated with 0.01 mL of a 48±2 hour old suspension of test organism. The inoculum was spread evenly over the carrier surface. The carriers were dried for 40-42 minutes at 33.7-34.2°C (which is a slightly longer time than the 30-40 minutes specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Each carrier was sprayed with the product for 2-3 seconds from a distance of 6-8 inches from the carrier surface, and allowed to remain wet for 5 minutes at 22.1-22.6°C. Following exposure, individual carriers were transferred to 20 mL of Letheen Broth to neutralize. Subcultures were gently shaken (see protocol) as specified in the AOAC method. All subcultures were incubated for ~49 hours at 33.6-34.9°C (which is a slightly longer time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum count, dried recovery carrier count, test system verification (i.e., purity and identity), neutralizer efficacy, and sterility.

Note: Testing performed on April 23, 2008 exhibited contamination in all test systems. Thus, the results were invalid. Testing was repeated on May 7, 2008. See pages 15 and 23 and Appendix B of the laboratory study.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Dried Carrier Count (CFU/ carrier)
		Lot No. 1325-046	**Lot No. 1325-069**	
474759-03	*Staphylococcus aureus* Test Date: April 4, 2008 Test Date: April 30, 2008	 1/10 0/10	 0/10 ---	 $\geq 1.9 \times 10^6$ $\geq 1.07 \times 10^6$
	Salmonella enterica	0/10	0/10	$\geq 1.38 \times 10^5$
	Pseudomonas aeruginosa Test Date: April 4, 2008 Test Date: April 30, 2008	 0/10 ---	 1/10 0/10	 $\geq 1.02 \times 10^6$ $\geq 1.19 \times 10^6$
		Lot No. 1325-045	**Lot No. 1325-047**	
474759-04	*Staphylococcus aureus Salmonella enterica Pseudomonas aeruginosa*	0/10 0/10 0/10	0/10 0/10 0/10	$\geq 9.7 \times 10^4$ $\geq 9.6 \times 10^4$ $\geq 1.6 \times 10^5$
		Lot No. 1367-073	**Lot No. 1367-076**	
474759-05	*Staphylococcus aureus Salmonella enterica Pseudomonas aeruginosa*	0/10 0/10 0/10	0/10 0/10 0/10	$\geq 2.22 \times 10^6$ $\geq 3.3 \times 10^4$ $\geq 2.6 \times 10^5$
		Lot No. 1367-064	**Lot No. 1367-068**	
474759-06	*Staphylococcus aureus Salmonella enterica Pseudomonas aeruginosa*	0/10 0/10 0/10	0/10 0/10 0/10	$\geq 2.72 \times 10^6$ $\geq 1.66 \times 10^5$ $\geq 3.84 \times 10^5$
		Lot No. 1367-080	**Lot No. 1367-083**	
474759-07	*Staphylococcus aureus Salmonella enterica Pseudomonas aeruginosa*	0/10 0/10 0/10	0/10 0/10 0/10	$\geq 2.62 \times 10^6$ $\geq 3.5 \times 10^4$ $\geq 5.2 \times 10^5$

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/carrier)		
474759-01	*Staphylococcus aureus*	960-028	$< 2.00 \times 10^1$	1.66×10^5	>99.9
		960-030	$< 2.00 \times 10^1$	1.66×10^5	>99.9
		960-032	$< 2.00 \times 10^1$	1.66×10^5	>99.9
	Klebsiella pneumoniae	960-028	$< 2.00 \times 10^1$	6.92×10^4	>99.9
		960-030	$< 2.00 \times 10^1$	6.92×10^4	>99.9
		960-032	$< 2.00 \times 10^1$	6.92×10^4	>99.9
474759-02	*Staphylococcus aureus* Test Date: 11/13/07	1056-078	1.41×10^3	1.95×10^6	99.9
		1056-079	1.15×10^3	1.95×10^6	99.9
		1056-080	2.09×10^3	1.95×10^6	99.89
	Staphylococcus aureus Test Date: 12/11/07	1056-080	2.34×10^3	2.34×10^6	99.9

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/carrier)		
	Klebsiella pneumoniae	1056-078	6.61×10^2	1.48×10^6	99.9
		1056-079	2.51×10^2	1.48×10^6	99.9
		1056-080	4.79×10^2	1.48×10^6	99.9

VI CONCLUSIONS

A. Conclusions Regarding Use of the Product as a Disinfectant

1. The submitted confirmatory efficacy data (MRID 474759-03 through -07) do not support the use of the following alternate formulations of the product, Brace, as disinfectants against *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5-minute contact time at full strength:

Formula Number 1178-172 (Crisp Linen)
Formula Number 1338-027 (Unfragranced)
Formula Number 1338-016 (Summer Breeze)
Formula Number 1338-015 (Spring Waterfall)
Formula Number 1338-020 (Garden Mist) (Pure Air)

Complete killing was observed in the subcultures of all carriers tested against the required number of product lots. [Note that repeat testing was conducted with one formulation (i.e., Formula Number 1178-172) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* to evaluate for false positives.] Neutralization confirmation testing showed positive growth of the microorganisms. Test system verification controls verified the identity of the challenge microorganisms. The sterility controls did not show growth. **Viability controls were not run as specified in the AOAC method**; however, dried recovery carrier controls showed positive growth of the microorganisms.

Numerous AOAC method deviations were cited regarding culture preparation, carrier drying, and subculture incubation. The culture preparation deviation (i.e. coarse filtered) is considered significant, and warrants rationale from registrant explaining the deviation from the method and feasibility. Briefly, the data submissions (page 7 of MRID No. 474759-03, 04, -05, -06, and -07) state that "any modifications to the published procedure are described in detail in the Procedure Description sections of the final report." These deviations (culture preparation, carrier drying time, and subculture incubation) were not included in the Protocol Deviation sections (pages 16, 15, 15, 15, 15 of the study MRIDs cited above). Additionally, **the registrant must provide rationale for the utilizing the Hard Surface Carrier Test method to the filtration step, and not continuing beyond this step (i.e. development of standard curve, etc.)**. Lastly, the Hard Surface Carrier Test method was the basis of the filtration step, however this method was not mentioned as a reference.

Rationale provided by Reckitt Benckiser (Diane Bosenberg, emailed on September 29, 2008):

"As per our conversation, I am responding back to your email from September 22, 2008 regarding the AOAC method deviations cited in the efficacy data presented for support of the Amendment on EPA Registration Number 777-99, OPP Decision Number D-397979.

As discussed, these deviations have been in place in our Microbiology laboratory for many years (some as far back as 10 years). They have been disclosed in our protocols and final reports during this time without issue. Also, they are explained in detail in our Microbiology SOPs which have been reviewed during our EPA audits without comment. The original registration for EPA Registration Number 777-99 was done in the same manner and was approved. Considering that these deviations to the AOAC method are standard practice and are disclosed in all of our documentation, we do not consider them deviations that need to be explained in the Protocol Deviations section.

In addition, as you know, the Germicidal Spray Products as Disinfectants method is currently under review and is being updated through US EPA (in conjunction with AOAC) and industry collaboration with the intention of harmonizing the language to be in line with the Use-dilution Method as revised and published in 2006. The test parameter changes are to include ranges where appropriate for things such as time and temperature. As revised, these modifications are more in line with Reckitt Benckiser standard practices as reported in MRID Numbers 474759-03, 04, 05, 06 and 07.

The deviations you cite in your email include: culture preparation, carrier drying and subculture incubation. This document will provide you with additional information on each of these points.

Culture Preparation:

The culture preparation information regarding the coarse filtration step is contained in our protocol under the section titled "Preparation of Test Culture / Addition of Organic Load". The specific directions state to "Pour the test culture through a sterile funnel containing coarse filtration medium to remove any particulates." Our standard practice is to use sterile glass wool as the filtration medium. Our SOP states:

> *Prior to the addition of organic soil, the test organism is coarse filtered through glass wool. In many laboratories, it is common practice to allow the test organism to sit prior to testing to allow for any extraneous organic matter to settle, and to be separated from the test organism that will be used for testing. In our laboratory, coarse filtering the test organism is performed to provide this result. Coarse filtering has not been shown to cause any change to the test organism, and inoculum and dried recovery control counts are routinely within the expected and acceptable ranges.*

This method of culture preparation removes any particulates, organism clumping and is a way to remove the pellicle from *Pseudomonas aeruginosa* cultures without the need for additional manipulation. We routinely see consistent and reproducible dried carrier counts using this technique (i.e. 10^5 to $10^{6/7}$). As an additional step in the test, the organism is verified for growth characteristics, purity and identity.

The current AOAC method 961.02 (Germicidal Spray Products as Disinfectants) states to

> "Thoroughly shake 48 h nutrient broth cultures of *S. choleraesuis* and *S. aureus* and let settle 10 min."

Taking this and the ongoing method revision, if you were to follow the methods Testing Disinfectants against *Salmonella choleraesuis* (955.14) or Testing Disinfectants against *Staphylococcus aureus* (955.15) for test culture preparation, the directions state to:

> "Using a Vortex-style mixer, mix nutrient broth test cultures 3 – 4 s and let stand 10 min at room temperature before continuing. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask ..."

The current AOAC method 964.02 (Testing Disinfectants against *Pseudomonas aeruginosa*) states:

"The pellicle from the 48 – 54 h cultures must be removed from the broth before mixing on a Vortex mixer either by decanting the liquid aseptically into a sterile tube or by gently aspirating the broth away from the pellicle using a pipet. ... Using a Vortex-style mixer ..."

We maintain that our method of filtration of the culture through sterile glass wool is equivalent to allowing the cultures to sit undisturbed, removing the upper portion and using that for testing purposes.

Carrier Drying and Subculture Incubation:

The carrier drying information is contained in our protocol in "Drying the Inoculated Test Surface". The specific directions state to

"Place the petri dishes containing the inoculated slides into a 35±2.5^{0}C incubator, and dry for 40 – 42 minutes."

The subculture Incubation information is contained in the protocol in "Incubation". The specific directions state

"The target temperature and duration for incubation of the test materials is 35±2.5^{0}C for a minimum of 46 hours."

Our SOP states:

Incubation temperatures will be 35 ± 2.5°C when the method specifies 37°C. Incubation temperature of 25 ± 2.5°C or 30 ± 2.5°C will be used for assays using fungi or other test systems requiring lower temperatures. Incubation duration will be over two or more nights. Incubators used in this test method are set and controlled at a set temperature range of 35 ± 2.5°C, 30 ± 2.5°C or 25 ± 1°C for optimal cultivation of organisms. These temperature ranges are monitored by the REES Series II PC Environmental Monitoring System (refer to SOP NO. M/V 78). The AOAC Germicidal Spray Products Method does not provide an incubation duration range. A precise 48 hour incubation is too rigid a time to read test results. Incubation over two nights is acceptable when growth controls are positive for bacteria. Fungi testing will require a longer incubation period according to the growth requirements of the organism being tested and can also be determined by sufficient growth of controls. Extended incubation provides a tougher criteria for the test substance, since sub-lethally injured organisms would have longer to recover and multiply. Extended incubation is acceptable as long as the subculture media does not evaporate or otherwise deteriorate, preventing observation of results.

The incubation time for the test materials is more specific in the protocol (i.e. ≥46 hours) than stated in the SOP (i.e. over two or more nights). The drying and incubation times are recorded in the raw data as time start and time end so the exact duration can be ascertained and reported.

When performing these methods, it is apparent that it is impossible to be as exact as the method specifies (i.e. 48 hours at 37°C). This necessitated the addition of ranges for time and temperature as stated in Reckitt Benckiser's SOPs. The time and temperature ranges for drying and incubation were determined by our laboratory to be optimal for the test organisms. Considering the expanded temperature range and drying and incubation time of the revised methods, our standard procedures are more in line with the method modifications and standard industry practices.

To ensure accuracy, all of the Microbiology laboratory equipment is monitored for environmental conditions using the REES Series II PC Environmental Monitoring System. This system allows for more precise data recording and collection throughout any given time period / study. The REES is a computerized system where through a series of probes which are connected to a centralized computer system, accurate information can be obtained about the specific conditions of that equipment. The system has the ability to scan the equipment for the set parameters (i.e. temperature / humidity) every 1.6 seconds and then records that information. Currently the system is set to record the temperature of the incubators at 1 hour intervals. Since the system is continuously scanned, if the temperature deviates from the set range, the system begins recording the temperature every minute until the parameters are once again met. This way we can monitor how long a temperature excursion occurred and determine its impact on the study. Most laboratories record temperatures using mercury thermometers at specific times of the day which does not allow for temperature fluctuations to be captured and is only a moment in time for a study that incubates over the course of several days.

In conclusion, all of the changes that we have made over the years have been done with much internal scrutiny and deliberation. I understand that you are putting these submissions under additional scrutiny due to the ATP program and industry inquiries. As I indicated to you, we intend to disclose our deviations during the workshop being planned between US EPA and industry. We stand behind our deviations as good science and have always thought of these deviations as ways to move the methods forward to current day technologies."

The registrant must submit confirmatory data consistent with the AOAC Germicidal Spray Products test (i.e. without the filtration step). The Agency has determined that utilizing the coarse filtration for particulate removal is a significant deviation. As this is a long-standing practice at Reckitt-Benckiser, the Agency will require the submission of confirmatory data for these bacteria.

B. Conclusions Regarding Use of the Product as a Sanitizer on Soft, Non-Food Contact Surfaces

1. The submitted efficacy data (MRID 474759-01) do not support the use of the product, Brace, as a spot treatment sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae* on soft, cotton weave fabric, non-food contact surfaces in the presence of a 5% organic soil load for a 30-second contact time at full strength. The alternate formulation, Formula Number 677-180, was used in efficacy tests. Although a bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes (actually 30 seconds), the carrier quantitation controls did not meet the inoculated control count specified in the ASTM method (i.e., at least 7.5×10^5 surviving organisms). [The carrier quantitation controls did meet the laboratory acceptance criterion of 2.0×10^4.] At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 474759-02) support the use of the product, Brace, as a spot treatment sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae* on soft, polyester/acrylic fabric, non-food contact surfaces in the presence of a 5% organic soil load for a 30-second contact time at full strength. The following alternate formulations were used in efficacy testing: Formula Number 1056-010B, Formula Number 1056-010C, and Formula Number 1056-054A. A bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes (actually 30 seconds). [Note that repeat testing was conducted with one formulation (i.e., Formula Number 1056-010C) against *Staphylococcus aureus*.] The carrier quantitation controls met the inoculated control count specified in the ASTM method (i.e., at least 7.5×10^5 surviving organisms). At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

Note: The "Conclusions" section of this report identifies an AOAC method deviation with regard to culture preparation. This deviation appears to be acceptable.

VII RECOMMENDATIONS

1. The confirmatory data do not support the use of the following alternate formulations of the product, Brace, as disinfectants on pre-cleaned, hard, non-porous surfaces against *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* for a 5-minute contact time at full strength:

Formula Number 1178-172 (Crisp Linen)
Formula Number 1338-027 (Unfragranced)
Formula Number 1338-016 (Summer Breeze)
Formula Number 1338-015 (Spring Waterfall)
Formula Number 1338-020 (Garden Mist) (Pure Air)

2. The proposed label claims that the product, Brace, is effective in sanitizing soft, non-food contact surfaces against *Klebsiella pneumoniae* and *Staphylococcus aureus* for a 30-second contact time at full strength. This claim is acceptable for synthetic fabrics, as it is supported by the submitted data. Proposed label directions indicate that use of the product as a fabric sanitizer is for spot treatment (2" x 2" area). As discussed in the "Conclusions" section of this efficacy report, this claim is not acceptable for cotton fabrics. The label must reflect limited use sites for spot soft surface sanitization (polyester/acrylic fabrics).

3. Claims throughout the proposed label imply use of the product on large soft surface areas, not "spot treatment" of surface areas suggested in the Agency's response to Item# 1 in the letter dated June 10, 2008. The proposed label must be revised, as appropriate.

4. The following revision is required on the proposed label:

- Under the "Special Instructions ..." section on page 8 of the proposed label, change "Hepatatis B" to read "Hepatitis B."

5. The Agency's requests to delete the claim "Kills 99.9% of odor-causing bacteria, even on soft surfaces" remains.

6. Remove the following claim for the proposed label, as it is not supported by efficacy data,

Starts to kill on contact

7. The claims, "Prevents mold and mildew from coming back" requires an "efficacy-supported" time frame.

8. The claim, "Fights the Flu virus" requires the statement the descriptor "on treated surfaces".

9. The use of the term "safe" is not acceptable.